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Research report

Impact of repeated stressor exposure on the release of corticotropin-releasing hormone, arginine-vasopressin and bombesin-like peptides at the anterior pituitary

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ABSTRACT

Repeated exposure to stressors was reported to increase the expression of arginine-vasopressin (AVP), especially in corticotropin-releasing hormone (CRH) neurons co-expressing AVP, within the hypothalamus. This may increase the potential for subsequent stressor-elicited enhancement of hypothalamic–pituitary–adrenal (HPA) functioning as these peptides synergistically stimulate pituitary ACTH secretion. Likewise, members of the bombesin (BB) family of peptides (including its mammalian analogues gastrin-releasing peptide (GRP) and neuromedin B (NMB)) stimulate the release of ACTH and may play a role in the mediation and/or modulation of the CRH stress response. In the present investigation, chronic stressor exposure (daily restraint over 14 days) was associated with increased co-expression of CRH and AVP at the median eminence. In addition, *in vivo* interstitial levels of anterior pituitary AVP, GRP and NMB (but not CRH) were elevated following chronic stressor exposure. Basal pituitary corticosterone levels, in contrast, were unaffected by chronic stressor exposure. Following consumption of a highly palatable snack, interstitial levels of CRH, GRP, NMB and corticosterone (but not AVP) were elevated at the pituitary; however, a cross-sensitization was not apparent among rats previously exposed to the stressor and then provided with the snack. As the CRH, AVP and BB-like peptide systems have been associated with altered anxiety and depressive symptoms, the sustained peptidergic alterations observed in the chronically stressed rats may have implications for the development of these stressor-related disorders.

1. Introduction

Stressor exposure activates the hypothalamic–pituitary–adrenal (HPA) axis, characterized by the secretion of ACTH into systemic circulation and the subsequent release of glucocorticoids [1,2]. Depending on the nature, intensity, duration and frequency of the stressor, the HPA axis may show habituation (blunted ACTH secretion) or sensitization (enhanced ACTH secretion) [3,4]. In addition, cross-sensitization may occur as in the case when repeated homotypic stressor exposure produces an exaggerated HPA axis response upon subsequent exposure to a heterotypic stressor [5].

Although the underlying neuronal mechanisms mediating HPA axis plasticity are not fully understood, there is evidence that at least some of the persistent effects of stressors may involve long lasting changes within corticotropin-releasing hormone (CRH) neurons originating in the paraventricular nucleus (PVN) of the hypothalamus that co-express arginine-vasopressin (AVP) [6,7]. Indeed, both CRH and AVP produced in the PVN are co-released (upon stressor exposure) from nerve terminals in the median eminence to synergistically stimulate the release of ACTH from the anterior pituitary [8–11]. Importantly, following a chronic stressor regimen, increased stores of AVP are present within CRH neurons in the median eminence [7,12–14]. It is thought that increased AVP availability might result in greater peptide release upon subsequent challenge, and hence together with CRH, would promote augmented pituitary ACTH secretion [7,13].

Corticotropin-releasing hormone, although considered the primary regulator of the HPA axis [1,2,8], is also released at both hypothalamic and amygdaloid sites in response to spon-

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taneous food ingestion [15–17]. Given that defensive behaviors are incompatible with those associated with feeding, it follows that those systems associated with stressor reactivity should be linked, directly or indirectly, with those subserving reductions of consummatory behavior [15,18,19]. Indeed, it has been demonstrated that appetitive or rewarding stimuli can activate the HPA axis, although it is unclear whether this reflects a response to arousal or whether food ingestion acts like a metabolic stressor [18,20].

In addition to CRH, bombesin-like peptides (BLPs), best recognized for their ability to suppress food intake (satiety peptide) [21–26], also act to moderate the stress response [27]. In this respect, gastrin-releasing peptide (GRP) and neuromedin B (NMB), mammalian analogues of bombesin (BB), are present in all major components of the HPA axis, including the hypothalamus, pituitary and adrenal gland [28–33]. Administration of BB or GRP activates the HPA axis as reflected by the increased release of ACTH and corticosterone [34–37]. This effect is attenuated by pretreatment with CRH antiserum or a receptor antagonist [34,35,37], suggesting a functional linkage between these two peptidergic systems. Moreover, like CRH, the release of BLPs is elevated in response to both palatable food ingestion and stressor exposure [15].

In view of the presumed effects of chronic stressor exposure on CRH and AVP within the median eminence, coupled with findings that sensitization and cross-sensitization effects may be induced by stressors, in the present investigation, we assessed whether a chronic stressor regimen would be accompanied by altered CRH and/or AVP availability at the anterior pituitary, the presumed target for these peptides. Indeed, as the anterior pituitary represents a major node where central nervous system signaling gets translated into endocrine responses, we felt it would be salient to measure potential paracrine and hormonal fluctuations at this level. In addition, as GRP and NMB have been implicated in the stress response and in modulation of HPA functioning (perhaps through interactions with CRH) we also assessed their availability at the anterior pituitary in response to chronic stressor exposure. Finally, to assess whether the chronic stressor proactively influenced the later response to an appetitive stimulus, peptidergic and corticosterone changes were evaluated at the anterior pituitary in response to palatable snack ingestion.

2. Experimental procedures

2.1. Animals

Individually housed male Sprague-Dawley rats (weighing between 325 and 400 g), obtained from Charles River (St. Constant, Quebec) were maintained on a 12-h light/dark cycle (with lights on at 6:00 a.m.), in a temperature (23 °C) and humidity (60%) controlled room and had free access to food and water. All experimental procedures were approved by the Animal Care Committee of the University of Ottawa, and met the guidelines provided by the Canadian Council on Animal Care as well as the "Principles of laboratory animal care" (NIH publication no. 86–23, revised 1985).

3. Experimental protocol

Rats (n = 8–11/group) were randomly assigned to one of two groups and exposed to either no stressor treatment or to a chronic restraint regimen (20 min daily restraint for 14 days; restraint comprised of placing the rat in a plastic cone-shaped bag with a small opening at the front and tightly securing the bag around the base of the tail). Following the chronic restraint group's last stressor treatment, rats from both groups were anaesthetized (60 mg/kg pentobarbital; i.p.) and 20 gauge guide cannulae (plugged with removable stainless steel stylettes) stereotaxically implanted at the anterior pituitary. The placement coordinates for the anterior pituitary were 5.0 mm posterior to bregma, 0.9 mm lateral to the midline and 0.5 mm dorsal to the sphenoid bone [38]. The guide cannulae for push–pull perfusion (custom made Derelin™ pedestals) were anchored to the skull surface using four stainless steel screws and acrylic dental cement. After a 5-day recovery period, animals were transferred to individual test cages for a 48 h period to acclimate them to the test environment.

During this 21 day period (14 days of stressor exposure and 7 days post-surgery) all rats (controls and those exposed to repeated restraint) were introduced and stabilized on intake to a Graham wafer snack (~3.5 g of Christie's Honeymaid®), so that upon its introduction (at approximately 11:00 a.m. daily), rats immediately approached and consumed this palatable snack within a 10 min time period. During the acclimatization phase, rats had free access to rat chow and water.

On the test day (i.e., following the 48 h acclimation period), the stylet within the guide cannula was replaced with a push–pull probe aimed at the anterior pituitary. After a 1 h period, which was previously shown to be a sufficient for stabilization [15], three baseline dialysate samples were collected followed by the presentation of the Graham wafer snack (~3.5 g of Christie's Honeymaid®) to which animals had previously been acclimated. Commencing immediately after snack ingestion (which occurred to approximately 11:00 a.m.), five further dialysate samples were collected and used for determination of peptide and hormone levels. The samples (150 μl) were collected at 10 min intervals, immediately frozen on dry ice and stored at −80 °C until radioimmunoassay (RIA) analysis.

3.1. Pull–pull perfusion

The push–pull probe consisted of an outer (or pull) stainless steel cannula (protruding 0.4 mm beyond the end of the permanent guide cannula) and an inner (or push) cannula (glass silica) protruding 0.2 mm beyond the end of the pull cannula and into the anterior pituitary. Probes were connected by polyethylene tubing (PE-20 and PE-10) to two independent, pre-equilibrated peristaltic pumps (Minipuls 3, Gilson, Middleton, WI), via a dual channel swivel assembly (Instech Laboratories, Horsham, PA). Kreb's ringer phosphate (KRB) solution consisting of (in mM): 145 Na^+, 2.7 K^+, 1.35 Ca^+, 1.0 Mg^{2+}, Cl^- and 0.1% BSA, was perfused through the push cannula at the rate of 15 μl/min.

3.2. Histology

Following completion of the dialysis experiment, rats received an overdose of pentobarbital and India ink (1 μl) was then delivered through the push–pull probe (into the perfusion site) for verification of probe placements. The pituitary gland was removed and the placement verified visually under a dissecting microscope. Only the data from rats with confirmed position at the anterior pituitary were used in the data analyses.

3.3. Radioimmunoassay (RIA)

A solid-phase RIA procedure was employed for the detection and quantification of CRH, AVP and BLPs (GRP and NMB) [39]. Corticosterone was also measured in the perfusates using a microadaptation [40] of the commercial RIA kit (MP Biomedicals, Solon, OH). Briefly (for CRH, AVP, GRP and NMB detection) protein A/G (Calbiochem Corp; La Jolla, CA) coated Immunilon-4 well plates (Dynatec Laboratories Inc., Chantilly, VA) were coated with CRH, AVP, GRP or NMB antibody (1:100,000 final dilution) for 2 h at room temperature. The plates were then rinsed three times with wash buffer. The standards (reconstituted in KRB solution; ranging from 0.05 to 250 fmol for CRH; 0.0025–125 fmol for AVP; 0.25–512 fmol